NZY T7 RNA Synthesis kit

Catalogue number:

MB35301, 50 reactions

Description

NZY T7 RNA Synthesis kit was designed for the *in vitro* production of RNA transcripts from any DNA template subjected to the control of a T7 promoter sequence. *In vitro* transcription is performed by an engineered version of phage T7 RNA polymerase. This enzyme displays a high specificity for the T7 promoter allowing an effective transcription of the DNA strand of interest from the promoter sequence. NZY T7 RNA Synthesis kit can be used to transcribe linearized plasmid templates or DNA templates generated by PCR.

All kit components were optimized to carry out fast and efficient transcription of RNA. This kit is formulated to provide high yields of RNA transcripts. Yields of approximately 50-80 µg of RNA from 0.5 µg DNA template may be expected from a 2 hours reaction as described below. The resulting RNA transcripts present high integrity and the required quality for a variety of downstream applications, such as RNA splicing studies or RNA-protein interaction experiments.

To maintain integrity of generated RNA transcripts, the T7 Enzyme Mix includes a Ribonuclease Inhibitor. This protein will effectively protect the synthesised RNA transcripts from degradation by contaminating RNases resulting from different sources, such as plasmid templates or others.

Storage temperature

Store all kit components at -20 °C in a freezer without defrost cycles. Stability can be extended by storage at -80 °C. NZY T7 RNA Synthesis kit components are stable up to the expiry date specified with the product.

Kit components

Component	Amount
T7 Enzyme Mix	50 µL
10× Transcription Reaction Buffer	100 µL
10 mM ATP	50 µL
10 mM CTP	50 µL
10 mM GTP	50 µL
10 mM UTP	50 µL
Linear DNA Control*	10 µL

* Provided for 3 experiments. The control template DNA is a 6043 bp linearized plasmid which codes for a 600 nt RNA transcript.

Protocol for *in vitro* RNA synthesis

NZY T7 RNA Synthesis kit was optimized to produce RNA transcripts using unmodified ribonucleotides. Nevertheless, this kit is compatible with capped RNA synthesis or RNA synthesis using modified ribonucleotides. The standard protocol for *in vitro* transcription is presented below.

Standard *in vitro* Transcription

Before you start using this protocol, please carefully read the Important Notes section below.

After mixing and quickly pulsing the reaction components, place T7 Enzyme Mix and ribonucleotides solutions on ice but **keep the 10× Transcription Reaction Buffer at room temperature.**

Note: Some components present in the 10× Transcription Reaction Buffer can co-precipitate the template DNA if the reaction is assembled on ice.

1. Set up the transcription reaction **at room temperature** by adding the bellow mentioned components in the order presented in the following table:

Nuclease-free water	up to 20 µL
10× Transcription Reaction Buffer	2 µL
10 mM ATP	1 µL
10 mM CTP	1 µL
10 mM GTP	1 µL
10 mM UTP	1 µL
Template DNA*	0.5 µg
T7 Enzyme Mix	1 µL

* For control reaction, use 3 µL of Linear DNA Control (0.5 µg)

2. Mix thoroughly and centrifuge briefly to collect all drops.
3. Incubate at 37 °C for 2 hours.
   Note: For short transcripts (<0.3 Kb), incubate the transcription reaction at 37 °C for 4 hours or more.
4. (Optional) *Procedure for removing DNA template*:
   If the template DNA will interfere with the downstream application of the RNA transcript, proceed with a DNase digestion once the transcription reaction is finished as following:
   Add DNase (not provided, but make sure that it is RNase-free) plus the respective reaction buffer directly to the transcription reaction; mix and incubate for 15 minutes at 37 °C. Stop DNase activity by adding 1 µL of EDTA at 0.5 M.
5. After the transcription reaction, proceed with purification of synthesised RNA and/or analysis of integrity, length and yield of the transcript on agarose gel.

Important notes

RNase contamination

An RNase-free working environment is essential to obtain high quality RNA and a successful *in vitro* transcription. For this reason, special precautions should be taken to avoid RNase contamination, such as:

- Always wear gloves; change gloves whenever you suspect of a contamination;
- Use RNase-free tubes and pipet tips;
- Designate a special area and equipment dedicated for RNA work.

All components of NZY T7 RNA Synthesis kit were tested to ensure the absence of RNases.

DNA Template

Any linearized plasmid or PCR products that contain a T7 promoter region may be used as template for *in vitro* transcription using the NZY T7 RNA Synthesis kit. The sequence of the T7 promoter recognized by T7 RNA polymerase is indicated in Figure 1.

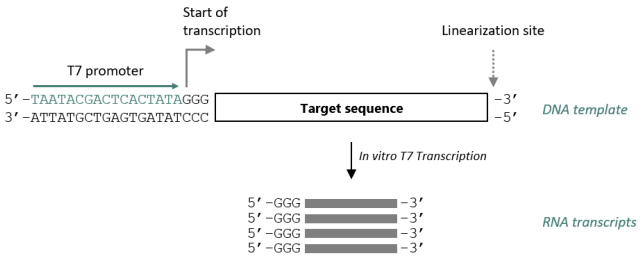

Figure 1. *In vitro* transcription from the T7 promoter.

The orientation of RNA synthesis (sense or antisense) depends on the orientation of the T7 promoter relative to the sequence of interest. Thus, for sense RNA transcripts, the sequence of interest must be placed downstream of the T7 promoter (see Figure 1).

Plasmid templates

The success of *in vitro* transcription using the NZY T7 RNA Synthesis kit depends on the quality and linearization of plasmid templates, since these two parameters affect the quality and quantity of synthesised RNA transcripts. Plasmid templates need to be linearized before the *in vitro* transcription reaction to allow the production of RNA transcripts with a defined length. Since T7 RNA polymerases are highly processive enzymes, circular plasmid templates are not recommended and will generate long and heterogeneous RNA transcripts. Although the *in vitro* transcription reaction will stop when T7 RNA polymerase recognizes a loop or hairpin termination region, its efficacy will depend on the type of terminator. Therefore, when using plasmid templates to generate RNAs with a defined length, it is highly recommended to digest the DNA at the 3´-region where transcription is aimed to stop.

Hence, it is highly recommended to linearize the plasmid template by restriction digesting the DNA at a position located downstream of the insert to be transcribed (Figure 1). Preferentially, DNA template should be double-digested upstream and downstream the region that needs to be transcribed. The use of restriction enzymes which generate blunt ends or 5' overhangs are preferred related to restriction enzymes that produce 3' overhangs. If using the last enzymes, then the linearized plasmid ends can be made blunted using Klenow Fragment of DNA polymerase I (Cat. No. MB009) prior to transcription.

PCR templates

DNA templates generated by PCR and containing a T7 promoter can be effectively transcribed using the NZY T7 RNA Synthesis kit. PCR products should be examined on an agarose gel (to estimate concentration and to confirm the expected size) before their incorporation in the *in vitro* transcription reaction. We recommend purifying PCR products to obtain better transcription yields.

Amounts of DNA template

We recommend using 0.5-1 µg of any DNA template in a 20 µL transcription reaction.

Quality control assays

Purity

The T7 Enzyme Mix components are >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining.

Nucleases assay

All components of the kit are tested for DNases and RNases contamination, using 0.2-0.3 µg of pNZY28 plasmid DNA and 1 µg of RNA, respectively. Following incubation at 37 °C during 14-16 hours for DNases assay and 1 hour for RNases assay, nucleic acid integrity is evaluated by agarose gel electrophoresis.

Functional assay

The kit is functionally tested in an *in vitro* transcription reaction. The result must be 80 µg of intact RNA transcript produced from 0.5 µg of linearized DNA template.

V1901

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assays	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
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